Development of Standard Procedures for a Simple, Rapid Test to Determine Wheat Color Class[1]

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ABSTRACT

Growing conditions, kernel characteristics, and genetics affect wheat kernel color. As a result, red and white wheats sometimes cannot be differentiated by visual examination. Soaking wheat kernels in a sodium hydroxide solution enhances the difference in color; red wheat turns a darker red, and white wheat turns straw-yellow. Previously, when NaOH was used for wheat determination of color class, only a visual assessment was made under arbitrary conditions, many times not suitable for field work. In the present work, visible reflectance spectroscopy and visual assessments were used to optimize NaOH (2 mL/g of wheat) soak time (10 min), concentration (5*M* or 20%), and temperature (60°C). The optimal procedure will provide users who are not laboratory trained with inexpensive, safe procedures to definitively assign wheat color class in the shortest time in field locations. Calibration and prediction of several wheat cultivars using partial least square regression were used to validate the optimal test procedure. The test differentiated even rain-bleached wheat and cultivars that were difficult to classify visually. No distinct correlation occurred between predicted color value and the number of red genes.

Red and white wheats should be kept segregated because they have different end uses. Both hard red and hard white wheats are used for bread, but white wheat is also used for Asian noodles. Until recently, red and white wheat were grown in separate regions of the United States. Thus, field inspectors grading wheat at one location generally knew the expected color class of samples received. However, with the rapid change in production from hard red wheat to hard white wheat expected in the hard winter wheat growing areas in the United States (to take advantage of potential export markets), many elevators likely will be receiving both wheat classes. Wheat kernel color is influenced by the number of red genes present (Metzger and Silbaugh 1970; Flintham 1992); environmental conditions (Quartley and Wellington 1962; DePauw and McCaig 1988), and kernel characteristics such as endosperm texture (Coles and Wrigley 1976). Thus, identifying the color class of wheat may not be straightforward.

Methods for determining color class include official visual identification; visual identification after soaking in potassium hydroxide (KOH) and bleach heated to 80°C (USDA 1997); visual identification after soaking in sodium hydroxide (NaOH) (Chmelar and Mostovoj 1938); colorimeters (Chen et al 1972; Bason et al 1995); machine vision (Neuman et al, 1989); visible and near-infrared (NIR) spectroscopy (Hawk et al 1970; McCaig et al 1992, 1993; Delwiche and Massie 1996; Dowell 1997, 1998; Wang et al 1999); and electrophoretic analysis (Lookhart and Wrigley 1995). Procedures using colorimeters, machine vision systems, spectrometers, or electrophoresis can improve classification accuracy over visual identification, but they can require calibration, trained personnel, significant initial costs, and considerable time. Visual classification of unsoaked kernels under visible light can be deceptive because some red wheats appear white and vice versa. Soaking in heated bleach improves visual classifications but poses safety risks. However, soaking kernels in sodium hydroxide is relatively safe and inexpensive, requires minimal user training, has never been reported to fail, and reduces subjectivity in visual determinations of color class.

Although the NaOH test has been used for many years, the procedures have not been optimized. For example, reported soak times range from 5 min to 2 hr (Chmelar and Mostovoj 1938; Quartley and Wellington 1962; Kimber 1971; Lamkin and Miller 1980; DePauw and McCaig 1988). Sample sizes range from 25 kernels in 25 mL of NaOH (Quartley and Wellington 1962) to 1 kernel in 1.5 mL (Lamkin and Miller 1980). Some researchers used surfactants to facilitate kernel immersion (DePauw and McCaig 1988). Also, some researchers conducted tests at room temperature, whereas Lamkin and Miller (1980) heated the solution to 55°C to speed the color change.

Before a standard test is recommended for widespread use to determine wheat color class, procedures should be developed that are safe, rapid, and inexpensive and give definitive results. Users of this procedure may not necessarily be laboratory trained personnel. When using the NaOH test, inexperienced users can draw erroneous conclusions if the color change is interpreted too soon or too late. Establishing standard procedures would ensure a more consistent interpretation of results. Thus, the objective of this research was to establish standard procedures for testing wheat using NaOH or other chemicals that will result in definitive classification of red and white wheats in the shortest time.

MATERIALS AND METHODS

Samples

Table I lists the wheat cultivars used in this study. The set consisted of red and white wheats in a range of shades and colors, cultivars with a known number of red genes, and bleached samples from three different harvest dates with additional rainfall in between. Red wheat samples with known red genes and pure white wheat samples were provided by the USDA-ARS National Small Grain Collection Laboratory (Aberdeen, ID) and the USDA-ARS Hard Wheat Quality Laboratory (Wang 1997); the American White Wheat Producers Association (Atchison, KS); and the Federal Grain Inspection Service (FGIS) of USDA-Grain Inspection, Packers and Stockyard Administration. Additional samples were available from previous studies (Dowell 1997; Wang 1997). Scabby kernels of red and white wheat were hand-picked under magnification of samples that were apparently heavily scab-infested. Scabby kernels could easily be identified by a shriveled, pale, and thinner than normal appearance. There was no difference between scabby kernels from both the red and white cultivars as both appeared bleached.

Optimization of NaOH Test

NaOH concentration. A solution (20.0% w/v) was prepared by dissolving 50 g of NaOH (>98%, pellets) in 250 mL of distilled

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TABLE I
Wheat Cultivars Used in NaOH Test[a]

Red Wheat		White Wheat	
Calibration Set	**Prediction Set**	**Calibration Set**	**Prediction Set**
Karl92[b,e]	Akron[b]	Betty[b,e]	Nu Plains[b,e]
Jagger[b,e]	Quantum[b]	Rio Blanco[b,e]	White Chief[b,e]
Larned[b,e]	Jagger[b,e]	K-196[b]	Betty[b,e]
Tam107[b]	Karl92[b,e]	Klasic[c]	Argent[c]
Tam105[c]	Diamant	Heyne[b,e]	Joel
Arapahoe[c]	Red Bobs	NuWest[b]	White Eagle
Ogallala[b]	Khurvof	Arlin[c]	Rely
Big Dawg[b]	Bezostaya	Snow White[c]	Hiller
Akron[b]	Brigrand	Nu Plains[b,e]	White Eagle[b]
Quantum XH-1888[b]	Grana	White chief[b,e]	BLM[d]
	Supreme		TOR[d]
	Vista[b]		
	Mardler		
	Apollo		
	Banco		
	Arin		
	Avalon		
	Bersee		
	2174[b]		

[a] First eight red and white wheat samples in calibration sets were used in partial least squares (PLS) analyses.
[b] 1999 samples grown in Kansas obtained from T. Joe Martin, KSU Agricultural Research Center, Hays, KS. Samples from harvest dates 6/26/1999, 7/5/1999, and 7/9/1999 with rain between harvest dates.
[c] Samples provided by FGIS.
[d] 1999 samples from American White Wheat Producers Assoc., Atchison, KS. Cultivar unknown.
[e] Different sample sets used in calibration and prediction categories.

water in a volumetric flask and then stored in a polyethylene bottle. A NaOH solution (20.0% w/v, ≈5*M*) was purchased commercially. Solutions of 0.5, 2.0, and 5.0% were prepared by diluting the 20% solution with distilled water in volumetric flasks and stored in polyethylene bottles. The solutions generally were kept capped, and only freshly prepared solutions were used for concentration-dependence studies. Kernels (≈30) of Tam107 (red) and Heyne (white) were soaked in 2 mL of NaOH at each concentration for 10 min at ambient temperature (22°C), and blotted dry; spectra were recorded. These samples represent the two color classes because several other cultivars examined behaved similarly by visual examination.

Soak time. For tests investigating the effect of soak time on near-infrared (NIR) absorbance, six kernels each of Betty, Larned, HRW103, and HWW1003 cultivars were placed individually in 1.5-mL NaOH solutions (2, 5, and 20%) in wells of a porcelain spot plate. Every 20 min, during a period of 2 hr, kernels were taken out of the solution and blotted dry with laboratory wipes. The NIR spectrum was obtained. In other tests, ≈60 kernels each of Rio Blanco (white) and Jagger (red) were soaked in 20% NaOH at 60°C for 5, 10, and 20 min and blotted dry. The NIR spectra were obtained. Untreated kernels were used as controls in both tests.

Temperature. Different types of heaters were used for studying the effect of the NaOH temperature on color classifications, depending on whether the sample was a single kernel, a few kernels, 1 g of wheat, or 10 g of wheat. For testing 1-g samples, an aluminum block heater (Reacti-Therm heating module, Pierce Chemical Co., Rockford, IL) accommodated 2.5-mL volume tapered, graduated heating module vials. The temperature was controlled using the coarse and fine Variac adjustments and was monitored with a thermometer immersed in the NaOH solutions. For temperature optimization tests, ≈60 kernels each of Rio Blanco (white) and Jagger (red) were soaked in 4 mL of 20% NaOH at 22, 41, 55, and 70°C for 10 min and blotted dry. The NIR spectra were obtained. In other tests, 30 kernels of each cultivar were soaked in 2 mL of NaOH and heated to the desired temperature. Excess NaOH solution was drawn off with a Pasteur pipette, and wheat kernels were blotted dry with laboratory wipes and dried on a filter paper.

For testing single kernels, a porcelain spot plate was set on a hot plate, and individual kernels were soaked in 1 mL of NaOH in the cavities of the spot plate. The temperature of the hot plate was controlled with a Variac. The temperature of the solution was monitored with a T-type filament thermocouple (Cu–CuNi) thermometer (Omega, Stamford, CT).

Effect of other solvents. Other solvents in addition to NaOH were investigated to determine whether they could improve color differentiation. Organic solvents tested were certified ACS grades. Saturated solutions of NaOH and KOH in ethanol, dimethyl sulfoxide (DMSO), 2-propanol, and acetonitrile were prepared by stirring 1 g in 20 mL of the solvent for 1 hr.

Color reversibility. To determine whether the NaOH-induced color change could be reversed, wheat kernels from cultivars of red (Tam107, Larned, and Karl92) and white (Betty, Heyne, Argent, and Klasic) wheats were soaked in 20% NaOH for 10 min at 60°C and dried for 30 min. These kernels then were soaked in 3*M* HCl at 22 and 60°C.

Procedure for field-testing wheat color with NaOH. A 600-mL beaker containing 450 mL of water was immersed in a water bath, and heated to 60°C using a hot plate or a common hot pot (soup heater) with a Variac adjustment to control temperature. Then 20 mL of 20% aqueous NaOH solution in a screw-cap Pyrex tube 20 × 150 mm was equilibrated to 60°C for 10 min. A 10-g sample of wheat was added to the hot NaOH solution, and the mixture was heated further for 10 min. Wheat kernels were filtered with a Buchner funnel with no filter paper, washed twice with 2 mL of water, transferred to a qualitative filter paper, and allowed to dry. A common coffee filter cone also suffices for filtering kernels in field applications. Color photographs of seven known cultivars of wheat samples before and after the test were used to assist in classifications.

Instrumentation

A diode array NIR spectrometer (Perten Instruments, Springfield, IL) was used to collect spectra from single wheat kernels before and after NaOH soaking. Baseline spectra were taken with the empty black bucket (that normally holds the sample wheat kernels), which gave a flat baseline in the entire spectral range. The trough sides of the bucket were at 45° angles from vertical. Although white ceramic-type materials typically are used for reference readings, the fixed test apparatus could not accommodate that material. Only information from the visible spectrum (400–750 nm) was used in this research. However, absorbance data in the 400–490 nm range were discarded

due to excessive noise. Kernels were placed in the black viewing bucket with tweezers to minimize errors from varying orientations. Kernels generally were placed with crease down and germ end toward the observer. Touching the kernels was avoided to prevent skin contaminants from affecting spectra or NaOH tests. White light illuminated a single kernel through a fiber optic bundle 8 × 60 mm at a 45° angle to the sample bucket. A fiber optic bundle 2 × 90 mm carried the kernel reflectance to the diode array. Fifteen spectra were collected from each kernel and averaged to reduce noise. Data were recorded in 5-nm increments. The spectra were stored on a hard disk for subsequent analysis.

Spectra Analysis for Optimization and Validation Tests

Spectra were analyzed by measuring tristimulus color space (*L**, *a**, and *b**) values as obtained by the diode-array spectrometer, by principal components analysis (PCA), or by partial least squares (PLS) regression (Martens and Naes 1989). The color space values are measures of the hue, saturation, and intensity of the kernel color. All PCA and PLS analyses were conducted with GRAMS/32 software (Galactic Industries Corp, Salem, NH) or Unscrambler (v. 7.5, Camo ASA, Corvallis, OR). These methods can factor out some variation in baseline offsets and varying kernel orientations.

Color space parameters. For tests investigating whether color space values could be used to determine the color class of soaked or unsoaked kernels, the kernels were prepared by soaking in 20% NaOH at 60°C for 10 min, removing excess moisture by blotting with a paper towel, and drying the kernels overnight on a filter paper. Visible NIR spectra of the dried kernels and unsoaked kernels were scanned with the crease side down (away from the light source) and the brush end pointing away from the observer. The cultivars tested include samples from successive harvest dates with rainfall in between, and scab-damaged kernels from both color classes. Color space values were computed from the average spectra from 60 kernels by first offsetting the spectra by a value of 1.2, which is the baseline shift caused by using a black reference instead of white reflectance material. The spectra were then transformed from log(l/R) to: % reflectance = $10^{(2-[\log 1/R])}$. *L**, *a**, and *b** values were then calculated from the % reflectance using Galactic/Grams32 software using a 2° standard observer and CIE *a* as the illuminant.

PCA analysis. The NIR spectra of 60 kernels each of Rio Blanco (white) and Jagger (red), unsoaked and soaked, were compared by PCA. Kernels were prepared by soaking in 20% NaOH solution for 10 min at 60°C and then blotted dry with laboratory wipes. The NIR spectra of 225 soaked and unsoaked kernels from several other cultivars also were obtained for PCA. This sample set, obtained from Federal Grain Inspection Service (FGIS), was an assortment of cultivars, many of which were of known color class and others assigned a color class during the test.

PLS analysis. Sixteen cultivars (eight red and eight white) of wheat were used in this study (Table I). Sixty kernels of each cultivar were soaked in 4 mL of 20% (5*M*) NaOH for 10 min at 60°C. Excess NaOH solution was removed using a disposable pipette. The kernels were blotted dry with laboratory wipes and air-dried. NIR spectra of kernels were obtained individually. Kernels were handled with tweezers only. If not dried at least for 30 min, kernels were sticky and difficult to orient and tended to leave residue in

TABLE II
Optimization of NaOH Soak Concentration and Soak Time

			Color Space Values[c]		
Wheat Cultivar	**NaOH (%)[a]**	**Time (min)[b]**	***L****	***a****	***b****
Tam107 (red)	0.0	· · ·	66.4	14.0	14.2
	0.5	· · ·	64.4	15.8	19.7
	2.0	· · ·	63.6	15.1	17.2
	5.0	· · ·	56.9	15.1	18.1
	20.0	· · ·	56.3	15.3	17.7
Heyne (white)	0.0	· · ·	69.1	11.1	11.7
	0.5	· · ·	66.0	11.7	14.3
	2.0	· · ·	66.7	11.9	16.1
	5.0	· · ·	65.6	12.0	15.9
	20.0	· · ·	61.6	12.4	16.5
Jagger (red)	· · ·	0	56.6	11.9	13.5
	· · ·	5	46.1	14.2	17.5
	· · ·	10	43.5	13.0	15.3
	· · ·	20	44.8	14.2	16.9
Rio Blanco (white)	· · ·	0	60.3	12.1	15.5
	· · ·	5	56.9	11.9	17.0
	· · ·	10	55.3	10.5	14.6
	· · ·	20	57.9	9.8	12.7

[a] 30 spectra averaged for each 15 min soak at 22°C, spectrum obtained immediately after blotting dry.
[b] 60 spectra averaged for soaks times with 20% NaOH at 60°C, spectrum obtained immediately after blotting dry.
[c] Color space values derived from spectra 490–750 nm.

TABLE III
Partial Least Squares (PLS) Parameters[a] for NaOH Soak Optimization

Variation	**Cultivar**	***N*[b]**	**Actual Values**	**Factors[c]**	**ExpY-var[c]**	***r***	**RMSEC**	**Slope**	**Offset**
Temperature									
	Jagger (red)	298	22, 41, 55, 70°C	15	94.6	0.96	5.68	0.95	2.00
	Rio Blanco (white)	286	22, 41, 55, 70°C	12	95.7	0.98	5.07	0.96	1.61
%NaOH									
	Tam107 (red)	151	0, 0.5, 2, 5 20%	5	93.2	0.97	1.82	0.94	0.33
	Heyne (white)	152	0, 0.5, 2, 5 20%	11	94.0	0.97	1.67	0.95	0.28
Soak time									
	Jagger (red)	241	0, 5, 10, 20 min	8	90.1	0.95	2.24	0.91	0.82
	Rio Blanco (white)	244	0, 5, 10, 20 min	9	95.5	0.98	1.56	0.96	0.39

[a] Correlation coefficient (*r*), root mean square error of calibration (RMSEC); slope and offset of regression line refer to plot of actual vs predicted values.
[b] Number of spectra analyzed.
[c] Number of factors recommended and Exp Y-var = corresponding explained y-variance.

the sample bucket. The drying time also helped most cultivars of red wheat to darken, which provided better contrast with white wheat, particularly in the bucket under the visible NIR light.

All data were mean centered by calculating the average of all spectra in the training set and then subtracting the result from each spectrum. Spectral data were analyzed by the Grams/32 PLS model using the absorbance data in the 490–750 nm range at 5 nm intervals (x-data) and constituent values (y-data) of 1 for white wheat and 2 for red wheat. The number of factors reported was the minimum required to give the maximum number of correct classification (spectra of kernels with predicted values >1.5 for red wheats and <1.5 for white wheats). The NaOH soak spectra for 922 kernels from eight red and eight white cultivars were analyzed using a full cross-validation scheme.

Visible wavelengths of spectra of the samples listed in Table I were used to predict the color of individual kernels. It included samples whose color class was known, red wheats with different numbers of red genes, and some white cultivars that appeared red visually. Samples for this set were prepared in an identical manner to those for calibration.

RESULTS

NaOH Concentration

The average color space values for the NaOH concentration study were obtained from an average spectrum obtained from individual spectra of 30 kernels of each cultivar. The average and standard deviation values were calculated from the first 10 individual spectra for each cultivar and treatment used ($r \approx 10\%$). Conditions corresponding to the maximum of a^* and b^* would correspond to the optimum soak conditions (Table II). However, $L^*a^*b^*$ values are interrelated, and it is important to consider L^* values together with a^* and b^*, as well as the trends in $L^*a^*b^*$ values to choose the optimum conditions. Soaking in 20% NaOH produced the largest change in $L^*a^*b^*$ values. A decrease in L^* denotes darkening. Increases in a^* and b^* values correspond to increase in red and yellow colors, respectively. Note that red wheat has a lower L^*, but higher a^* value than white wheat corresponding to its darker and reddish appearance. Generally, rates of chemical reactions increase with concentration of the reactants. Similarly, the predicted values for 20% NaOH were more separated than the predicted values for other concentrations of NaOH (data not shown). The data for Tam107 converged more easily, as seen by the number of factors used, than data for Heyne (Table III), showing larger spectral changes in red wheat than in white wheat upon soaking in NaOH. This optimum concentration was good also for other cultivars examined visually. The solubility limit for NaOH is a little more than 50%, but the pH level does not increase proportionately, so we would not expect 50% to be much better than 20%. Furthermore, handling a 50% solution is inconvenient because of high viscosity and precipitate formation. Additionally, even at 20% NaOH (5M), pH > 14 and the solution has to be considered marginally unstable.

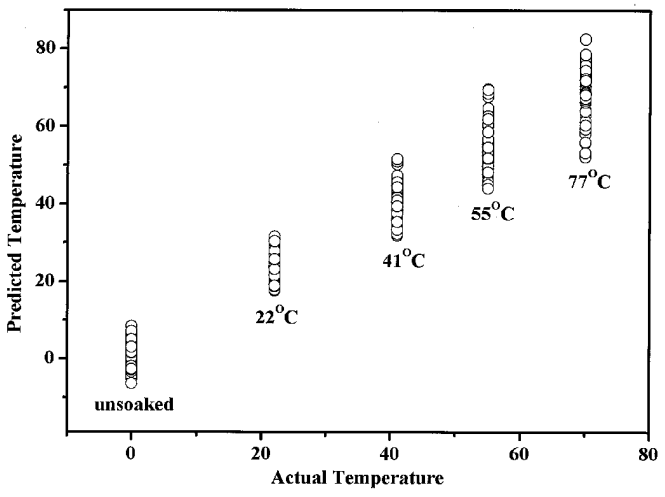

Fig. 1. Optimization of soak temperature (20% NaOH for10 min). Partial least squares (PLS) calibration developed using visible spectra (490–750 nm) of Rio Blanco kernels soaked in NaOH vs. actual soak temperatures. Soak temperature predicted from this model. Each circle corresponds to a single kernel of each cultviar ($n \approx 60$) at each temperature.

Soak Time

The color space values for the NaOH soaking time (Table II) study were obtained from an average spectrum obtained from individual spectra of 60 kernels of each cultivar. The average and standard deviation values were calculated from the first 10 individual spectra for each cultivar and treatment used ($r \approx 10\%$). The most pronounced changes in a^* and b^* values take place in 5 min. But the overall changes in $L^*a^*b^*$ values are not that different for 10 min soak, especially considering the trends in these values with respect to time. We preferred a 10-min soak when conducting the test on a 10-g scale (with no stirring) for better thermal equilibration. Soaking >10 min led to some bleeding of the yellow color in white wheat (Table II). With >20 min of soaking, the outer pericarp and seed coat tended to peel off of many cultivars, and starch gelatinization was also significant. The deprotonation was slow, perhaps because it had to take place under heterogeneous conditions on the seed coat beneath the outer pericarp, which includes a hydrocarbon layer (Bradbury et al 1956). Statistics for the PLS analysis of soak optimization are given in Table III.

Soak Temperature

Soaking at 22°C was not effective. For both red and white wheats, a yellow color formed on the outer pericarp. This yellow pigment partially masked the red pigment on the red wheat. At higher temperatures, this yellow color was extracted in solution, thus providing better differentiation of red and white wheats. A PLS analysis of the spectra (490–750 nm) with unsoaked kernels arbitrarily assigned 0°C was performed using leverage correction. The goal of this analysis was not to predict the temperature of NaOH soak, but rather to find a temperature that results in maximum difference from the unsoaked kernels. At the saturation point, color change was complete and we expected considerable overlap of the predicted y-values at various temperatures. The PLS analysis for Rio Blanco kernels is shown in Fig. 1. Note that the predicted y-data (temperatures) at 55 and 70°C show considerable overlap, indicating limiting color changes at these temperatures. Similar data for Jagger do not show saturation, but a progressive increase in the red color formed. Score plots from PCA of the visible spectral data for Jagger and Rio

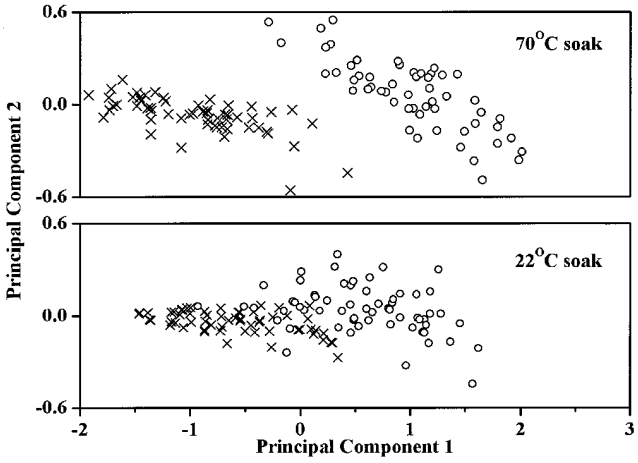

Fig. 2. Optimization of soak temperature. Principal component analysis (PCA) of 490–750 nm spectra of kernels of Rio Blanco white wheat (×) and Jagger, red wheat (O) in 20% NaOH for 10 min.

Blanco at 22 and 70°C soak temperature (Fig. 2) show that the difference in spectra of red and white wheat was enhanced more at 70°C than at 22°C. We observed that soaking wheat kernels at higher temperatures or soaking >10 min caused 1) peeling off of pericarp in some cultivars, 2) degradation of kernel color, 3) decomposition of the kernel to a gelatinous material, and 4) evaporation of water from the bath.

Effects of Other Solvents and Bases

Aqueous KOH was as effective as aqueous NaOH for color formation in red and white wheat cultivars, if the concentrations were considered in terms of molarity rather than percentage. It was also the most soluble of the other solvents tested. A 10% aqueous solution of tetrabutylammonium hydroxide, $(CH_3CH_2CH_2CH_2)_4NOH$ also caused white wheat to turn yellow and red wheat to turn yellow-brown. However, the difference in color was less pronounced than for aqueous NaOH soaking. Other bases such as pyridine (pH ≈ 9) and sodium carbonate (pH ≈ 12) did not cause color changes. Soaking 1.5 g of red or white wheat for 1 hr in nonaqueous solvents saturated with NaOH or KOH caused only slight color changes. In fact, the colors of Heyne and Tam107 were bleached slightly with KOH in DMSO, NaOH in acetonitrile, or NaOH in ethanol. Because only hydroxide (the strongest base in aqueous medium) was effective at concentrations >0.5% (0.125*M*) that gave pH > 13, and the compounds identified in the extract were phenolic, the color formation appears to be initiated by deprotonation of phenolic compounds. This may be the reason that other bases were not effective. The ineffectiveness of other solvents probably was due to relatively low polarity, ion-pair formation, and insufficient solubility of ionic hydroxides.

Reversibility

The reaction of HCl with NaOH-treated wheat was faster at 60°C than at 22°C. The HCl bleached some of the yellow and brown colors caused by the NaOH reaction. Successive NaOH and HCl treatments resulted in progressive loss of pigments. The yellow color of white wheats was more resistant toward HCl treatment than the brown color from red wheats.

The study of kinetics of color change in wheat kernels by monitoring absorbance as a function of NaOH soak time for various concentrations and temperatures was not successful. The total absorbance changes were fairly small; no new peaks were formed or original old peaks decayed by the NaOH soak. Difference between spectra of red and white wheats appeared to be maximum at 490–750 nm (Fig. 3). In this range, water does not absorb light energy, and no new peaks appeared in the spectra of either white wheats or red wheats after NaOH soaking. Also, baseline offsets were not uniform because of slight changes in orientation every time a kernel was taken out and replaced. Plots of absorbance versus time from surface reflectance measurements of NaOH-soaked wheat kernels showed no clear trend at any wavelength, rather than showing an exponential growth or decay. Thus, they were not useful for optimizing the conditions for the NaOH soak.

Validation of NaOH Test Procedures

The color space values for several red and white wheat cultivars, harvested at different dates after successive rainfall were obtained from an average spectrum from the individual spectra of 60 kernels of each cultivar. These color space values derived from spectra of individual kernels of red and white wheats harvested after successive rain events and of scab-damaged kernels, with and without soaking in NaOH, are given in Table IV. The average and standard deviation values were calculated from the first 10 individual spectra for each cultivar and treatment used ($r \approx 10\%$). Upon soaking, L^* values decreased and a^* and b^* increased for both red and white cultivars, but the changes in L^*, a^* and b^* values upon soaking in NaOH generally were smaller for white wheat. Note that the differences in $L^*a^*b^*$ values for red (average of several cultivars)

TABLE IV
Color Space Values Derived from Visible Spectra (490–750 nm) of White and Red Wheat Kernels, Soaked and Unsoaked in NaOH[a]

	Unsoaked			Soaked		
Wheat Cultivar[b]	***L****	***a****	***b****	***L****	***a****	***b****
White						
Betty-1	61.8	12.7	15.8	53.5	13.4	14.5
Betty-2	65.5	11.9	15.0	56.0	13.7	15.3
Betty-3	68.9	12.0	15.2	62.2	14.2	16.4
Heyne-1	65.7	11.5	13.9	61.1	13.6	15.9
Heyne-2	68.3	10.9	11.9	61.8	13.3	15.8
Heyne-3	69.9	11.2	12.3	65.9	14.0	17.5
White Chief-1	66.3	13.4	16.7	66.1	11.3	-1.3
White Chief-2	67.2	12.4	14.5	63.0	17.1	23.9
White Chief-3	73.6	13.5	15.3	66.0	16.6	18.8
Argent	62.5	13.0	13.9	56.0	15.4	17.1
Klasic	72.5	12.6	14.9	65.6	15.8	18.9
Rio Blanco-1	57.9	11.9	14.8	58.6	11.9	15.4
White (average)	66.7	12.3	14.5	61.5	14.2	15.7
Scabby (white)[c]	68.3	10.6	13.5	58.1	14.3	17.8
Red						
Tam107-1	66.9	14.2	15.8	52.0	19.1	20.4
Tam107-2	62.8	13.7	15.8	49.9	19.0	19.7
Tam107-3	69.2	15.2	17.5	55.4	20.6	21.1
Jagger-1	60.4	12.5	14.8	48.2	17.0	18.9
Jagger-2	65.7	12.7	13.9	52.6	15.4	12.5
Jagger-3	62.4	12.4	13.9	51.4	19.7	24.6
Karl92-1	62.3	12.6	14.7	52.8	16.6	19.2
Karl92-2	65.4	12.2	13.9	52.9	16.6	19.2
Karl92-3	68.4	13.0	15.1	55.2	19.1	23.2
Larned-1	61.9	13.9	17.0	46.5	17.5	20.4
Red (average)	64.5	13.2	15.2	51.7	18.1	19.9
Scabby (red)[c]	69.0	9.5	12.5	50.9	14.3	14.9

[a] Average spectra of 60 kernels; soaked kernels were dried overnight.
[b] 1,2,3 represent harvest dates 6/26/1999, 7/5/1999, and 7/9/1999 with rain between harvest dates.
[c] White scabby kernels were picked from infested Heyne and red scabby kernels were selected from an infested unknown red cultivar.

and white (average of several cultivars) wheat are substantially larger after NaOH soak. The *L*a*b** values also are consistent with red wheats developing a darker red and white wheats becoming yellow.

The red and white cultivars from successive harvest dates appeared more bleached after additional rainfall between those dates. For most cultivars, white wheats from harvest date 1 looked more reddish than red wheats from harvest date 3. However, in the NaOH soak test, white wheats always turned straw-yellow and red wheats always turned brown. Only a slight difference in the intensity of the colors occurred after the NaOH soak for wheat of either color class from different harvest dates. For unsoaked kernels, *L** values generally increased (brightening) and *a** and *b** values (red and yellow colors) decreased with successive harvest dates. Thus, unsoaked Karl92, which is genetically red, from harvest dates 2 and 3 appeared white, and Betty and White Chief, which are genetically white, from harvest date 1 appeared red. However, upon soaking in NaOH, their true color class became obvious by their appearance and their *L*a*b** values.

Scabby kernels from red and white wheats turned weakly red and straw yellow, respectively, upon soaking in NaOH, but some dark coloring was present. Severely scabby kernels with rosy pigment turned dark with NaOH treatment. Some of the natural red or white pigments may still be present in scabby kernels, although that may not be apparent before NaOH soaking. Scabby kernels from red and white wheats could not be distinguished before NaOH soaking either by visual appearance or by *L*a*b** values (Table IV). The color space values were higher for scabby kernels than for the healthy kernels before and after soaking. The difference in color space values between scabby red and scabby white kernels was larger after NaOH soaking than before.

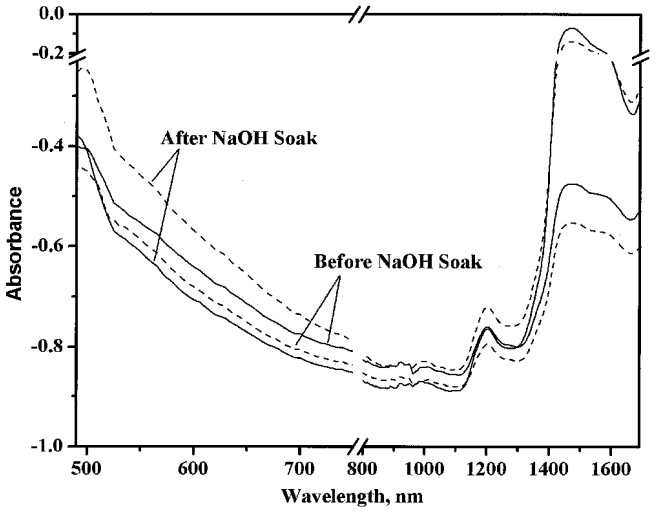

Fig. 3. Typical visible and near-infrared reflectance spectra of single kernels of white (——) and red wheat (- - - -) before and after NaOH soaking.

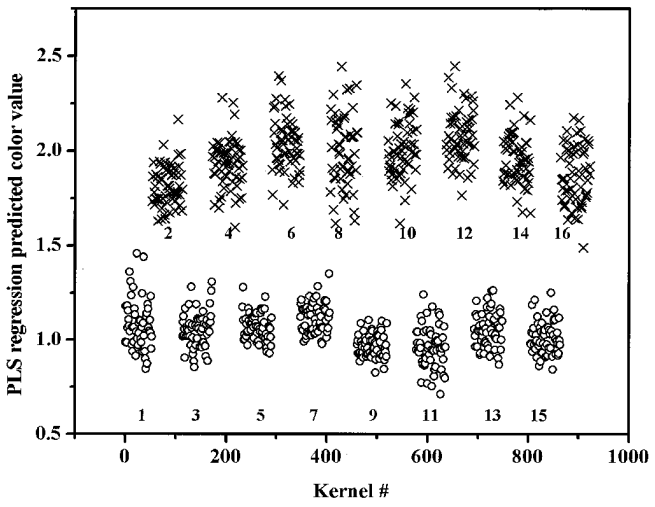

Fig. 4. Color classification of wheat soaked in NaOH. Partial least squares (PLS) regression of visible spectra (490–750 nm) of 16 cultivars (8 red, 8 white); y value for one kernel in each cultivar ($n \approx 60$): 1 = white (O), 2 = red (×).

TABLE V
Summary of Partial Least Squares (PLS) Color Calibration and Prediction

	Calibration[a]		Prediction[b]	
Parameters	r^2	**SECV**	**White Wheat**	**Red Wheat**
7 PLS factors	0.88	0.17		
12 PLS factors	0.91	0.15		
n			465	457
Mean y			1.04	1.96
SD y			0.11	0.16
Min			0.71	1.49
Max			1.46	2.45
Least square			1.08	1.93
%Correct			100	100

[a] Correlation coefficient (*r*), standard error of cross-validation (SECV).
[b] Constituent variation values (y-data). Color value 1 white wheat, 2 red wheat; *n* = number of kernels analyzed.

TABLE VI
Partial Least Squares (PLS) Predicted Values for Wheat Color Based on Visible Spectra After NaOH Soak[a]

	Predicted Values[b]			
Wheat Cultivar	***n***	**Mean**	**SD**	**Red Genes**
White				
Nu Plains	56	1.12	0.10	
Argent[c]	58	1.18	0.09	
White Chief	58	1.07	0.10	
White eagle	59	1.07	0.11	
Betty	47	1.16	0.13	
White eagle/Larned	63	1.10	0.11	
BLM[d]	60	1.01	0.12	
TOR[d]	60	0.84	0.08	
Joel	13	1.18	0.18	
Rely	7	1.03	0.08	
Hiller	7	1.07	0.10	
Scab-damaged	60	2.21	0.37	
Red				
2174	60	1.69	0.16	
Jagger	59	1.81	0.11	
Karl92	57	1.68	0.10	
Quantum XH-1888	58	1.83	0.12	
Akron	59	1.85	0.13	
Vista	58	2.06	0.12	
Diamant I	12	1.53	0.09	1
Red bobs	13	1.71	0.12	1
Mardler	15	1.82	0.11	1
Apollo	10	2.14	0.14	1
Grana	14	1.91	0.13	1
Supreme	15	2.07	0.24	1
Khurvof	25	1.79	0.08	2
Sperber	16	1.84	0.10	2
Bezostaya	14	2.01	0.14	2
Brigrand	10	1.72	0.09	2
Avalon	16	2.04	0.16	2
Bersee	19	1.93	0.31	2
Banco	10	1.94	0.10	3
Arin	16	1.92	0.13	3
Scab-damaged	60	2.08	0.56	

[a] Model in Figure 4.
[b] Color value 1 white wheat, 2 red wheat; *n* = number of kernels analyzed.
[c] Appears red but is actually a white wheat.
[d] Wheat label, not cultivar name.

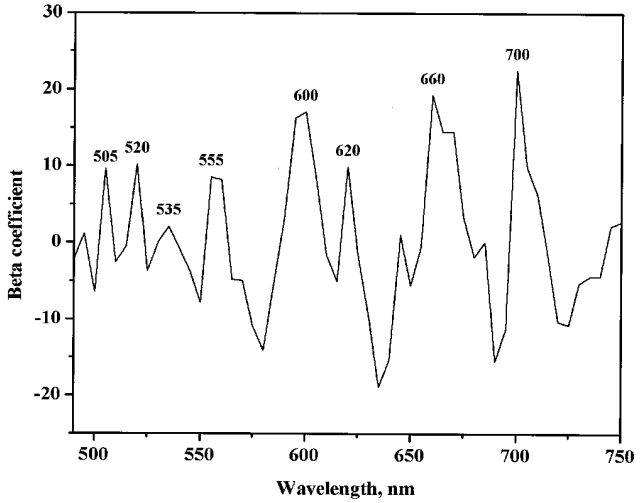

Fig. 5. β-Coefficients as a function of wavelength obtained in partial least squares (PLS) regression from Fig. 4.

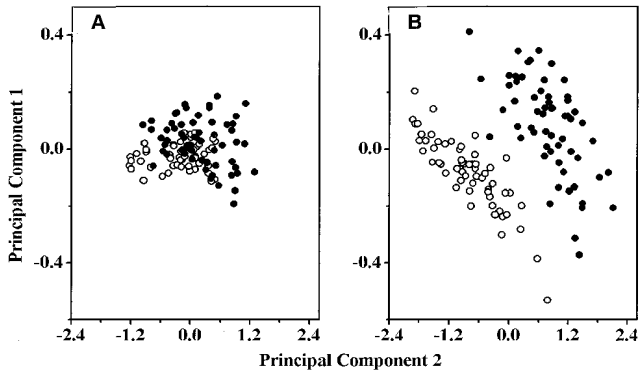

Fig. 6. Principal component analysis (PCA) of visible spectra (490–750 nm) of Rio Blanco white wheat (O) and Jagger red wheat (●). Each circle corresponds to a single kernel of each cultviar ($n \approx 60$) before (**A**) and after (**B**) NaOH soaking.

Classifying Wheat Using PLS

Results of PLS calibration and prediction are summarized in Tables V and VI and Fig. 4. Spectra from ≈60 kernels from each of 16 cultivars (8 red and 8 white) soaked in NaOH according to the standard procedure were analyzed by PLS. The plot of predicted color value for each kernel versus the kernel number (Fig. 4), shows clearly that 100% of the kernels were classified correctly after NaOH soaking. This is a modified plot of actual value versus the predicted value in which each data point corresponds to a kernel. There are two groups corresponding to red and white. The separation into other groups is not by cultivar but merely represents the order in which the kernels were scanned. A plot of the corresponding β-coefficients versus wavelength for NaOH-soaked kernels is shown in Fig. 5. Peak values of wavelengths are important for discrimination of red and white wheats.

The color value (white = 1 and red = 2) of each kernel was predicted with the Grams/32 PLS calibration model and the spectral data (490–750 nm) of 1,044 kernels soaked in NaOH. This prediction set also included cultivars with known red genes. All cultivars of red and white wheats were predicted correctly (Fig. 4), including the Argent wheat kernels that appeared red but actually were genetically white. Kernels of contrasting color classes were also predicted correctly. Of all the cultivars, only Karl92 (a popular red cultivar in Kansas) and Diamant did not turn very dark after NaOH treatment, but they were differentiated easily from all the white wheats. Jagger wheat, another popular Kansas cultivar, turned darker red than Karl92 but not as dark as some other cultivars upon NaOH soaking. Rain bleached the colors of wheat in their natural state but did not have much influence on the NaOH test. Bleached samples gave only slightly lower prediction values.

No correlation was observed between the prediction values and the number of red genes in red wheat (Table VI). However, analysis of dry single kernels of red and white wheats by single kernel NIR spectroscopy (400–1,700 nm) showed a correlation between the degree of red pigmentation and the number of red genes (Wang 1997).

Classifying Wheat Using PCA

Spectra from ≈240 kernels (120 soaked and 120 unsoaked) were analyzed by PCA. Results for Rio Blanco (white) and Jagger (red) wheat are shown in Fig. 6. Red and white wheats are clustered closer together with considerable overlap in the before soak set, whereas they are mostly separated in the after soak set. In each case, PC1 explains ≈98% of the variance in the data. The PCA analysis of *L*a*b** values gives similar results (data not shown).

DISCUSSION

The color of wheat kernels appears to be determined mostly by pigments in the seed coat and pigment strand (Bradbury et al 1956; Miyamoto and Everson 1958; MacMasters 1962; Evers and Bechtel 1988). Miyamoto and Everson (1958) identified catechin and catechin tannin as the precursors of brown pigment and showed a correlation between kernel color and the quantities of those precursors present in immature kernels. They also reported that the pigment occurs mostly in the seed coat rather than in the pericarp. McCallum and Walker (1990) suggested that trace levels of proanthocyanidins in the bran contributed to seed coat color in wheat. However, their findings were not substantiated by spectroscopy.

The reasons for the color change in red and white wheats treated with NaOH are not well understood. However, it is readily apparent from results reported here and previously by DePauw and McCaig (1988) that genetically red wheat contains pigments, probably in the seed coat, that turn brown when exposed to NaOH. These pigments are absent in genetically white wheat, so the change in color of white wheat to straw yellow after treatment with NaOH may be due to other compounds, possibly flavones and carotenoids (Simpson 1935), in the endosperm or seed coat that show through the transparent pericarp. Genetically white wheat cultivars that appear red likely have a reddish pigment in the endosperm or seed coat that either does not change color or becomes somewhat yellow upon treatment with NaOH. Genetically red wheats that appear white may contain low concentrations of pigments that impart color to kernels other than the pigments that react with NaOH and turn brown. Because all genetically red wheats contain the critical pigments that turn brown upon treatment with NaOH, the NaOH test determines the genetic color class independent of visual appearance before soaking.

CONCLUSIONS

An optimum procedure was developed for an NaOH soak test to determine wheat color class. Soak parameters such as time, temperature, concentration of NaOH, and the NaOH solution volume per amount of wheat sample were optimized (10 min at 60°C in 20% NaOH, 2 mL/g of wheat kernels, respectively) using a combination of visible reflectance spectra and visual examination. A test kit with the necessary equipment is being marketed commercially (Perten Instruments, Springfield, IL). The test can be used by personnel with no laboratory experience such as those at farm and commercial grain storage localities. PLS analysis of NIR data from ≈30 wheat cultivars soaked in NaOH validated the standard procedure by correctly predicting the color class, including the ones that were confusing before the NaOH soak. Chemical characterization of the NaOH color reaction with wheat surface pigment is still being pursued.

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LITERATURE CITED

Bason, M. L., Zounis, S., Ronalds, J. A., and Wrigley, C. W. 1995. Segregating red and white wheat visually with a tristimulus colour meter. Aust. J. Agric. Res. 46:89-98.

Bradbury, D., M., MacMasters, M. M., and Cull, I. M. 1956. Structure of the mature wheat kernel. II. Microscopic structure of pericarp, seed coat, and other coverings of the endosperm and germ of hard red winter wheat. Cereal Chem. 33:342-360.

Chen, C. Y., Skarsaune, S. K., and Watson, C. A. 1972. Relation of kernel color to wheat class and grade. Cereal Sci. Today 17:340-343.

Chmelar, F., and Mostovoj, K. 1938. On the application of some old and the introduction of new methods for testing genuineness of variety in the laboratory. Proc. Int. Seed Testing Assoc. 10:67-74

Coles, G. D., and Wrigley, C. W. 1976. Laboratory methods for identifying New Zealand wheat cultivars. N. Z. J. Agric. Res. 19:499-503.

Delwiche, S. R., and Massie, D. R. 1996. Classification of wheat by visible and near-infrared reflectance from single kernels. Cereal Chem. 73:399-405.

DePauw, R. M., and McCaig, T. N. 1988. Utilization of sodium hydroxide to assess kernel color and its inheritance in eleven spring wheat varieties. Can J. Plant Sci. 68:323-329.

Dowell, F. E. 1997. Effect of NaOH on visible wavelength spectra of single wheat kernels and color classification efficiency. Cereal Chem. 74:617-620.

Dowell, F. E. 1998. Automated color classification of single kernels using visible and near-infrared reflectance. Cereal Chem. 75:142-144.

Evers, A. D., and Bechtel, D. B. 1988. Microscopic structure of the wheat grain. Pages 47-96 in: Wheat Chemistry and Technology. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

Flintham, J. 1992. Grain color and sprout-resistance in wheat. Pages 30-36 in: Pre-Harvest Sprouting in Cereals. M. K. Walker-Simmons and J. L. Ried, eds. Am. Assoc. Cereal Chem.: St. Paul, MN.

Hawk, A. L., Kaufmann, H. H., and Watson, C. A. 1970. Reflectance characteristics of various grains. Cereal Sci. Today 15:381.

Kimber, G. 1971. The inheritance of red grain colour in wheat. Z-Pflanzenzucht 66:151-157.

Lamkin, W. M., and Miller, B. S. 1980. Note on the use of sodium hydroxide to distinguish red wheats from white common, club, and durum cultivars. Cereal Chem. 57:293-294.

Lookhart, G. L., and Wrigley, C. W. 1995. Variety identification by electrophoretic analysis. Pages 55-71 in: Identification of Food-Grain Varieties. C. W. Wrigley, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

MacMasters, M. M. 1962. Important aspects of kernel structure. Trans. ASAE 5:247-249.

Martens, H., and Naes, T. 1989. Multivariate Calibrations. John Wiley and Sons: Guildford, England.

McCaig, T. N., McLeod, J. G., Clarke, J. M., and DePauw, R. M. 1992. Measurement of durum pigment with an NIR instrument operating in the visible range. Cereal Chem. 69:671-672.

McCaig, T. N., DePauw, R. M., and Williams, P. C. 1993. Assessing seed-coat color in a wheat breeding program with a NIR/Vis instrument. Can. J. Plant Sci. 73:535-539.

McCallum, J. A., and Walker, J. R. L. 1990. Proanthocyanidins in wheat bran. Cereal Chem. 67:282-285.

Metzger, R. J., and Silbaugh, B. A. 1970. Location of genes for seed coat color in hexaploid wheat, *Triticum aestivum* L. Crop Sci. 10:495-496.

Miyamoto, T., and Everson, E. H. 1958. Biochemical and physiological studies of wheat seed pigmentation. Agron. J. 50:733-734.

Neuman, M. R., Sapirstein, H. D., Shwedyk, E., and Bushuk, W. 1989. Wheat grain color analysis by digital image processing. II. Wheat class discrimination. J. Cereal Sci. 10:183-188.

Quartley, C. E., and Wellington, P. S. 1962. Biochemical tests for seed identification. J. Natl. Inst. Agric. Bot. 9:179-185.

Simpson, A. G. 1935. A simple method for determining the "Yellowness" and "Grade" of wheat flours. Cereal Chem. 12:569-574.

USDA. 1997. Grain Inspection Handbook. II. Grain Grading Procedures. Grain Inspection, Packers and Stockyards Admin.: Washington, DC.

Wang, D. 1997. The determination of single wheat kernel color class using visible and near-infrared reflectance. PhD dissertation. Texas A&M University: College Station, TX.

Wang, D., Dowell, F. E., and Lacey, R. E. 1999. Single wheat kernel color classification using neural networks. Trans. ASAE 42:233-234.

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